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14. ABSTRACT

1-*N*-Methyl-5-(β-D-ribofuranosyl)uracil (1-*N*-methyl-pseudouridine) will be studied as a new imaging probe for cellular proliferation. The specific aims are: (1) to prepare and test 1-*N*-[^{3}H]methyl-pseudouridine in vitro in comparison with [2-^{14}C]thymidine, (2) to synthe- size 1-*N*-[^{11}C]methyl-pseudouridine, and (3) to examine 1-*N*-[^{11}C]methyl-pseudouridine in vivo using micro-PET imaging. This project is still in the early phase of development, consequent- ly, there are no findings, significant results or conclusions that can be reported at this time. Critical starting material (pseudouridine) has been obtained in order to begin the synthetic and radiochemistry development. In addition, 2'-deoxy-pseudouridine and pseudo- thymidine have been obtained in order to facilitate radiochemistry development for C-11 radiolabeling of 1-*N*-methyl-pseudouridine. Some of the pseudothymidine has been custom H-3 labeled which should provide additional biological information for this class of pseudo-nucleosides, and will be compared with that of 1-*N*-[^{3}H]methyl-pseudouridine in the future.

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Introduction

Rapid cellular proliferation is a key characteristic of malignancy that should be exploited for diagnostic imaging of patients. For the clinical management of breast cancer, there are consistent data indicating that biological markers for proliferation are good indicators of prognosis and can provide information regarding response to treatment (*1*). Consequently, an imaging agent that can distinguish high-grade lesions from benign or low-grade tumors, or detect high-grade transformation within a low-grade tumor would be invaluable for tumor characterization and treatment planning. Increased levels of uptake of a tumor proliferation specific probe should correspond with higher-grade malignancy or biologic aggressiveness (*2*). A number of 5-substituted derivatives of thymidine and 2'-fluoroarabinofuranosyl analogs of thymidine have been prepared with imaging radionuclides and are being studied as potential tracers for cellular proliferation (*3*).

The purpose of this research project is to study the efficacy of 1-*N*-methyl-5-(β-D-ribofuranosyl)uracil (1-*N*-methyl-pseudouridine) as a new imaging probe for cellular proliferation as applied to breast cancer. The reasoning behind this compound is that it closely resembles thymidine, but this structure has a C-C linkage between the sugar and base, rather than a C-N bond. The key advantage anticipated would be to ensure in vivo metabolic stability, and avoidance of the rapid catabolism characteristic of thymidine itself. Imaging with [^{11}C]thymidine is plagued with rapid degradation in vivo, generating many [^{11}C]labeled metabolites, which in turn makes image analysis very difficult (*4*).

Body

The objective is to prepare, and test biologically, radiolabeled 1-*N*-methyl-pseudouridine as an imaging probe for tumor proliferation. This material must be first synthesized in unlabeled form, then as a ^{14}C-labeled compound. 1-*N*-[^{14}C]Methyl-pseudouridine will be tested in vitro. Depending on the in vitro results, then will undertake to prepare the ^{11}C-labeled derivative for in vivo micro-PET (Positron Emission Tomography) imaging studies.

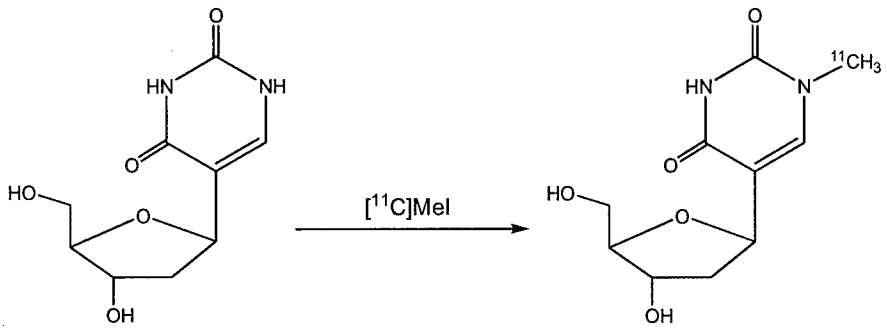
The first task is to prepare unlabeled 1-*N*-methyl-pseudouridine for chemical characterization and for use as a cold reference standard. The first limitation was that Sigma-Aldrich stopped producing pseudouridine which is the key starting material to prepare pseudouridine derivatives. I found MP Biomedicals, Inc did carry this compound, but I only bought 50 mg from them $526.95 to start with until I could find a less expensive source. Eventually, I found Berry & Associates, from whom I could obtain 1 g of pseudouridine for $1395.00. Now, I have sufficient starting material to proceed with the synthesis of 1-*N*-methyl-pseudouridine.

The next task is to prepare starting material to send to Moravek Biochemicals, Inc for preparation of 1-*N*-[^{14}C]methyl-pseudouridine. The problem that arose in this task is after detailed assessment of the ^{14}C-labeling issues, Moravek Biochemicals provided a final quotation that exceeded the budget established ($9000) for this task. They are requesting $16,850 to produce the 1-*N*-[^{14}C]methyl-pseudouridine. This information was not available at the time of grant submission as such custom work takes a lot of time to assess the potential work involved. Consequently, although less preferable, it was decided to pursue the ^{3}H-labeled version instead, which if I provide the unlabeled 1-*N*-methyl-pseudouridine (5 mg of material), then Moravek Biochemicals will produce the ^{3}H-labeled 1-*N*-methyl-pseudouridine for $3,400. This is the only feasible path to pursue. The planned work can be accomplished with the ^{3}H-labeled version instead of the ^{14}C-labeled. The ^{14}C compound is much more stable and has a longer shelf life than the ^{3}H version, but is unfortunately much more costly to produce.

Until the ^{3}H-labeled 1-*N*-methyl-pseudouridine is available, the in vitro cell work with W256 cell line cannot be performed. We maintain a stock of [2-^{14}C]Thymidine, which the tritiated compound will be compared against in the cell assays.

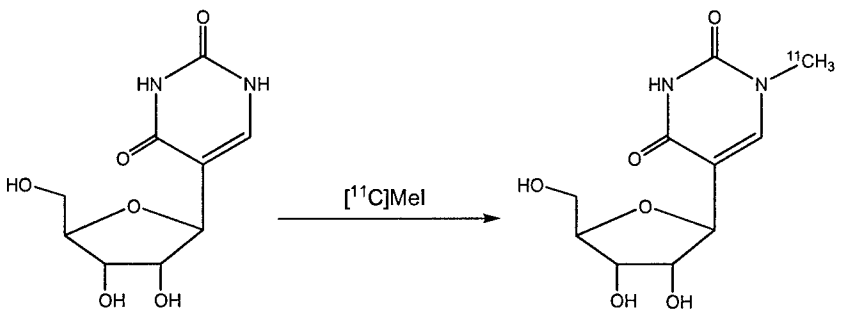
The other important task to pursue is to develop effective radiolabeling methodology that is suitable for use with ^{11}C. This effort begins with small scale, non-radioactive labeling studies. As a starting point for this task, it was decided to begin with methodology that has already been used for the labeling of pseudothymidine (*5*). All of the reagents have been obtained for this work to begin. In addition, it was decided that it would be best to duplicate the methodology for labeling pseudothymidine before applying it to pseudouridine, so some 50 mg of 2'-deoxy-pseudouridine and some standard pseudothymidine (both available from Berry & Associates, Inc) has been purchased to accomplish this goal.

^{11}C-Labeling of Pseudothymidine



2'-deoxy-pseudouridine [^{11}C]pseudothymidine

^{11}C-Labeling of Pseudouridine



pseudouridine 1-*N*-[^{11}C]methyl-pseudouridine

With the availability of some pseudothymidine in our possession, it was decided to have some of this material custom tritiated by Moravek Biochemicals, Inc. This was readily accomplished, and we now have [methyl-^{3}H]pseudothymidine in our hands to study by in vitro assay as well. This will provide some important data to compare with ^{3}H-labeled 1-*N*-methyl-pseudouridine in the future. [Methyl-^{3}H]Pseudothymidine will give us an early insight into the biological behavior of this class of pseudo-nucleosides, particularly with regard to in vivo stability and cell uptake characteristics.

Until the chemistry/radiochemistry challenges are worked out and some cell studies are performed, animal imaging studies cannot be attempted.

Key Research Accomplishments

There are no key research accomplishments to report yet, as this project is still in the early phase.

Reportable Outcomes

There are no reportable outcomes to present, as this project is still in the early phase.

Conclusions

There are no conclusions to be made at this time.

References

1. Ravaioli A, Bagli L, Zucchini A, Monti F. Prognosis and prediction of response in breast cancer: the current role of the main biological markers. *Cell Prolif.* 1998;31:113-126.
2. Britz-Cunningham SH, Adelstein SJ. Molecular targeting with radionuclides: state of science. *J Nucl Med.* 2003;44:1945-1961.
3. Mangner TJ, Klecker RW, Anderson L, Shields AF. Synthesis of 2'-deoxy-2'-[18F]fluoro-β-D-arabinofuranosyl nucleosides, [^{18}F]FAU, [^{18}F]FMAU, [^{18}F]FBAU and [^{18}F]FIAU, as potential PET agents for imaging cellular proliferation. *Nucl Med Biol.* 2003;30:215-224.
4. Conti PS, Alauddin MM, Fissekis JR, Schmall B, Watanabe KA. Synthesis of 2'-fluoro-5-[^{11}C]-methyl-1-β- D-arabinofuranosyluracil ([^{11}C]-FMAU): a potential nucleoside analog for *in vivo* study of cellular proliferation with PET. *Nucl Med Biol.* 1995;22:783-789.
5. Grierson JR, Shields AF, Zheng M, Kozawa SM, Courter JH. Radiosyntheses of labeled β-pseudothymidine ([C-11]- and [H-3]methyl) and its biodistribution and metabolism in normal and tumored mice. *Nucl Med Biol.* 1995;22:671-678.